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Sub-Lethal Effects of Herbicides on the Wolf Spider *Pardosa milvina*

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Abstract

We tested the sub-lethal effects of six commonly applied herbicides on juveniles of the agriculturally abundant wolf spider *Pardosa milvina*. We compared spider toxicological effects from herbicides that were freshly applied to soil, aged for 69 days indoors at room temperature, or aged for 69 days in a greenhouse with variable temperature, humidity, light, and evaporative water cycling. Field-collected juvenile *P. milvina* were exposed to one of eight herbicide treatments (atrazine, glyphosate, mesotrione, S-metolachlor, 2,4-D, dicamba, a combination of all six herbicides, or a distilled water control; N = 960, n = 40, across 24 treatments) and maintained for 49 days on the treated soil substrate. We recorded prey capture behavior, weight change, and growth rate across treatments. Mesotrione had particularly significant negative effects on feeding and weight gain. Mesotrione impaired prey capture latency and led to weight loss. We found significantly decreased molting frequency of spiders in the 2,4-D, S-metolachlor, glyphosate, and dicamba treatments relative to the control but this effect was not present in the greenhouse-aged soil treatments. Fresh and indoor-aged soil had similar effects while greenhouse-aged soil dampened most herbicide effects indicating photodegradation and/or temperature degradation of herbicides over the 69-day period. Our results show that some herbicides significantly impair feeding and growth rates in this agriculturally abundant predator with some effects detectable even with greenhouse-aged herbicide residues.

Introduction

- Modern agricultural practices have led to a dramatic increase in pesticide use, especially over the last few decades with the US consuming over 18% of all commercial pesticides worldwide and herbicides comprising two-thirds of that pesticide use (Atwood and Paisley-Jones 2017).
- Many of these herbicides are applied prior to crop growth (pre-emergence), so non-target ground-dwelling species are often directly exposed to chemicals that may have numerous unintended effects (Evans et al. 2010; Niedobová et al. 2019; Godfrey and Rypstra 2018).
- The breakdown time for different herbicides varies greatly, and some herbicides may persist for weeks or even months. Glyphosate, one of the most used, and as a result, most studied herbicides take 10-60 days to reach 50% breakdown (Thompson et al. 2000). Less common herbicides have not been studied extensively, and the variability in environmental and microbial conditions make breakdown effects even harder to predict.
- *Pardosa* is the second largest genus of spider, with species found in agricultural systems across six continents. *Pardosa milvina* is a ground spider that consumes ca. half a million insects per week per acre of corn or soybeans in Central Pennsylvania. Thus, the effects of herbicides on these arthropods may have a potentially significant impact on their efficacy as biocontrol agents in pest control (Reichert and Lockley 1984; Nyffeler and Breene 1987; Marshall et al. 2002).
- We quantified sublethal effects (i.e., feeding behavior, weight changes, and ecdysis) of commonly used herbicides on juveniles of the agriculturally relevant wolf spider *Pardosa milvina*.

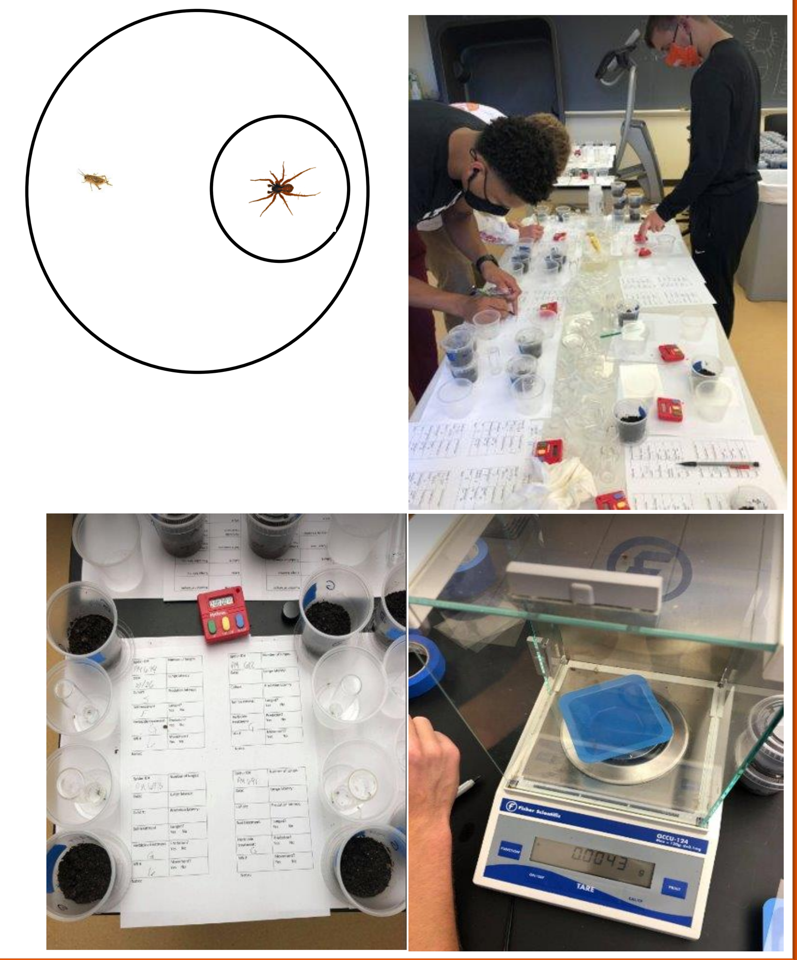
Questions

- **Does chronic herbicide exposure affect growth and behavior of the wolf spider *Pardosa milvina*?**
- **Does herbicide age and sun exposure affect toxicity to the wolf spiders?**

Methods

Feeding Trials

- A single *Pardosa milvina* spider was placed in a clean empty 473 ml plastic cup underneath a clear plastic vial. The container also had one 1st instar cricket (*Gryllodes sigillatus*).
- After a short acclimation period, the vial was lifted, and the time required to capture the cricket was recorded over a 20 minutes period.
- After the spider had captured the cricket or the trial time expired, the spider was fed 6 – 8 additional 1st instar crickets.
- All spiders were weighed prior to the experiment then once weekly for seven weeks.
- All molts found were recorded during the 49-day testing period.



Summary and Conclusions

- **Spider prey capture latency was generally slower across all herbicide treatments compared to the control treatments after one week of exposure (Figure 1, Table 1).**
  - All herbicide treatments experienced an increase in latency to capture relative to the control treatment, with mesotrione and combined treatments having the most pronounced effects.
  - The freshly-sprayed soil treatments experienced greater capture latency compared to the greenhouse and indoor-aged soil treatments across all treatment groups.
- **Some herbicides reduced molting frequencies of spiders (Figure 2, Table 2).**
  - Spiders exposed to the herbicides 2,4-D, glyphosate, S-metolachlor, and dicamba showed significantly fewer molts compared to controls and molting differences were greatest in the indoor-aged soil treatment.
- **We found significant differences in weight change over the 49-day period for greenhouse-aged soil across herbicide treatment (Figure 3, Table 3).**
  - By week five, mesotrione, s-metolachlor and combined herbicide treatments had significantly higher weights.
  - At week six, mesotrione and combined continued to have significantly higher weights than all other treatment groups.
  - Breakdown products of these herbicides, especially mesotrione, have different toxicological effects than the freshly-sprayed herbicides. Previous studies suggested positive survival effects of glyphosate and atrazine on adult *Pardosa* (Ward et al. in prep) but here we show negative growth effects of these same herbicides on juveniles.

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Results

Prey capture latency

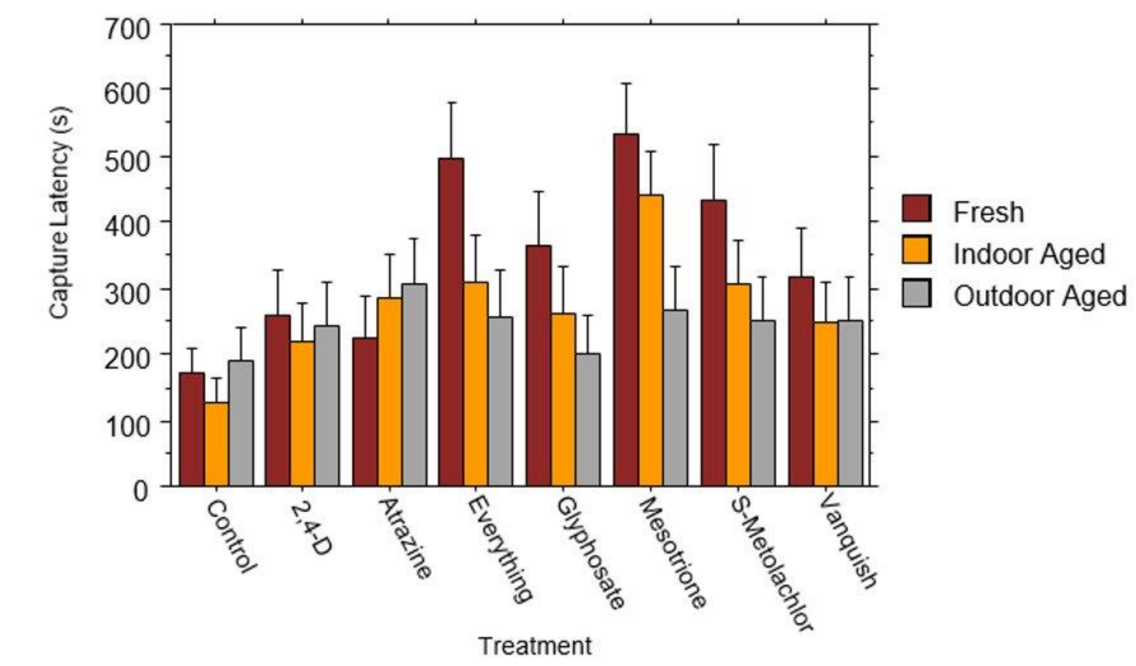


Figure 1. The average time (sec) to capture a cricket across each herbicide treatment and soil age after 7 days of exposure (N=960; n=40).

Treatment	F	P-value
Herbicide	3.846	0.0004
Soil Age	5.233	0.0055
Herbicide * Soil Age	0.987	0.4643

Table 1. Two-way ANOVA for the main effects and interactions between herbicide treatment and soil age on prey capture latency after 7 days of exposure.

Number of molts across herbicide and aged soil treatments

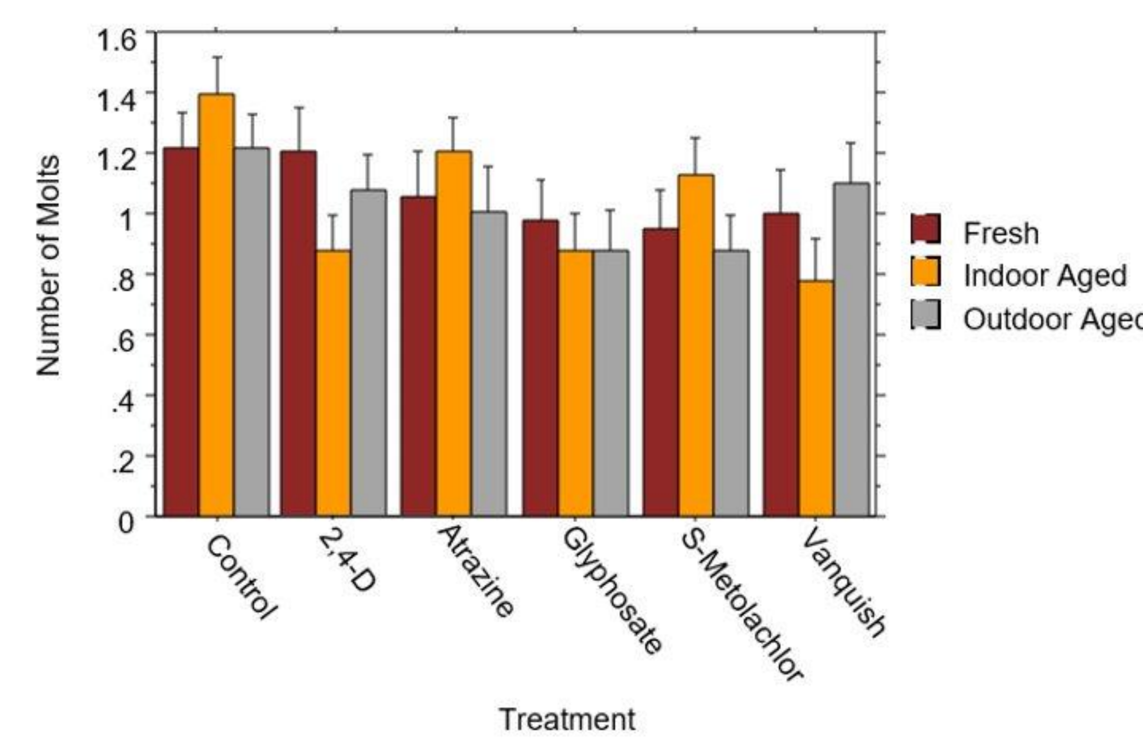


Figure 2: The number of molts for each treatment group over 49 days compared to the control. Mesotrione and combined herbicide treatments were omitted due to high mortality within the first two weeks (N=720).

Treatment	F	P-value
Herbicide	3.136	.0083
Soil Age	.156	.8554
Herbicide * Soil Age	1.098	.3606

Table 2. Two-way ANOVA for the effects of herbicide, soil age, and interactions on the number of molts the spiders went through.

Weight by time and herbicide treatment in outdoor-aged

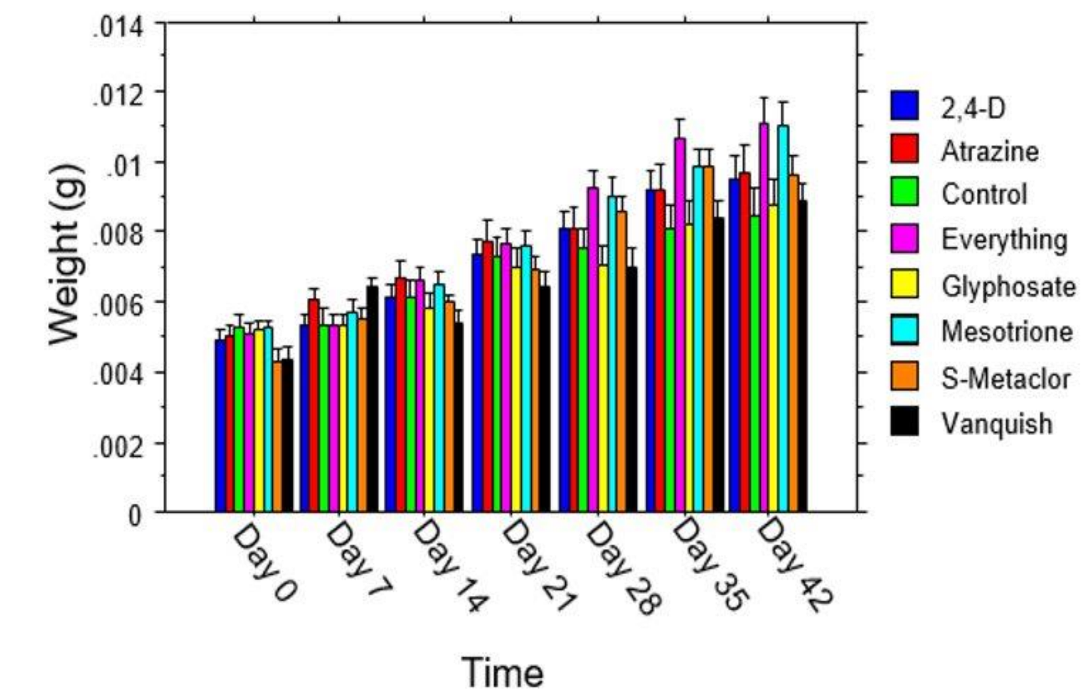


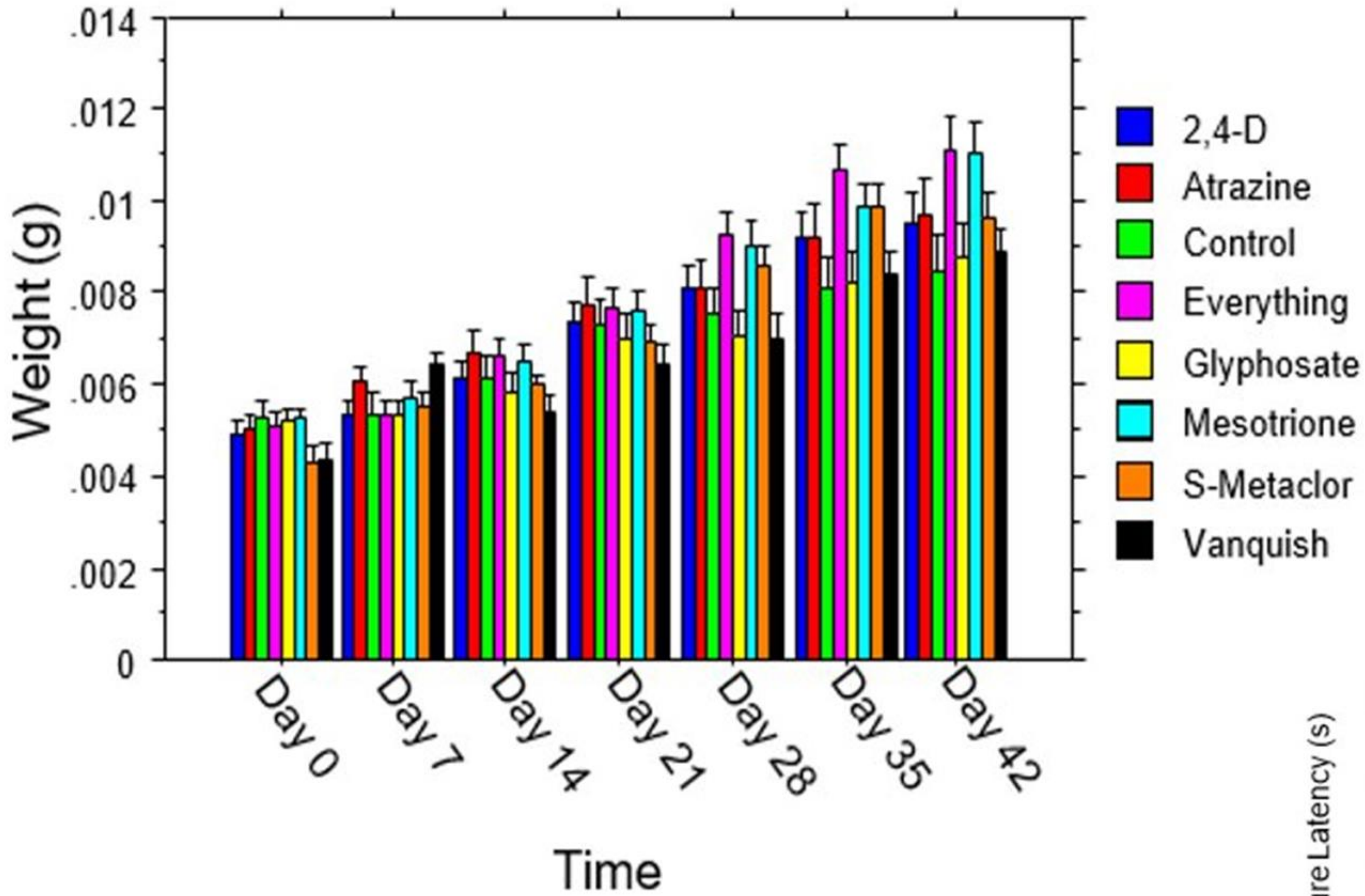
Figure 3: The weight (g) of spiders in each herbicide treatment over the 49 days of the experiment in the outdoor-aged soil treatment (N=360).

Treatment	F	P-value
Herbicide	1.14	.3382
Week	356.373	<.0001
Herbicide * Week	3.660	<.0001

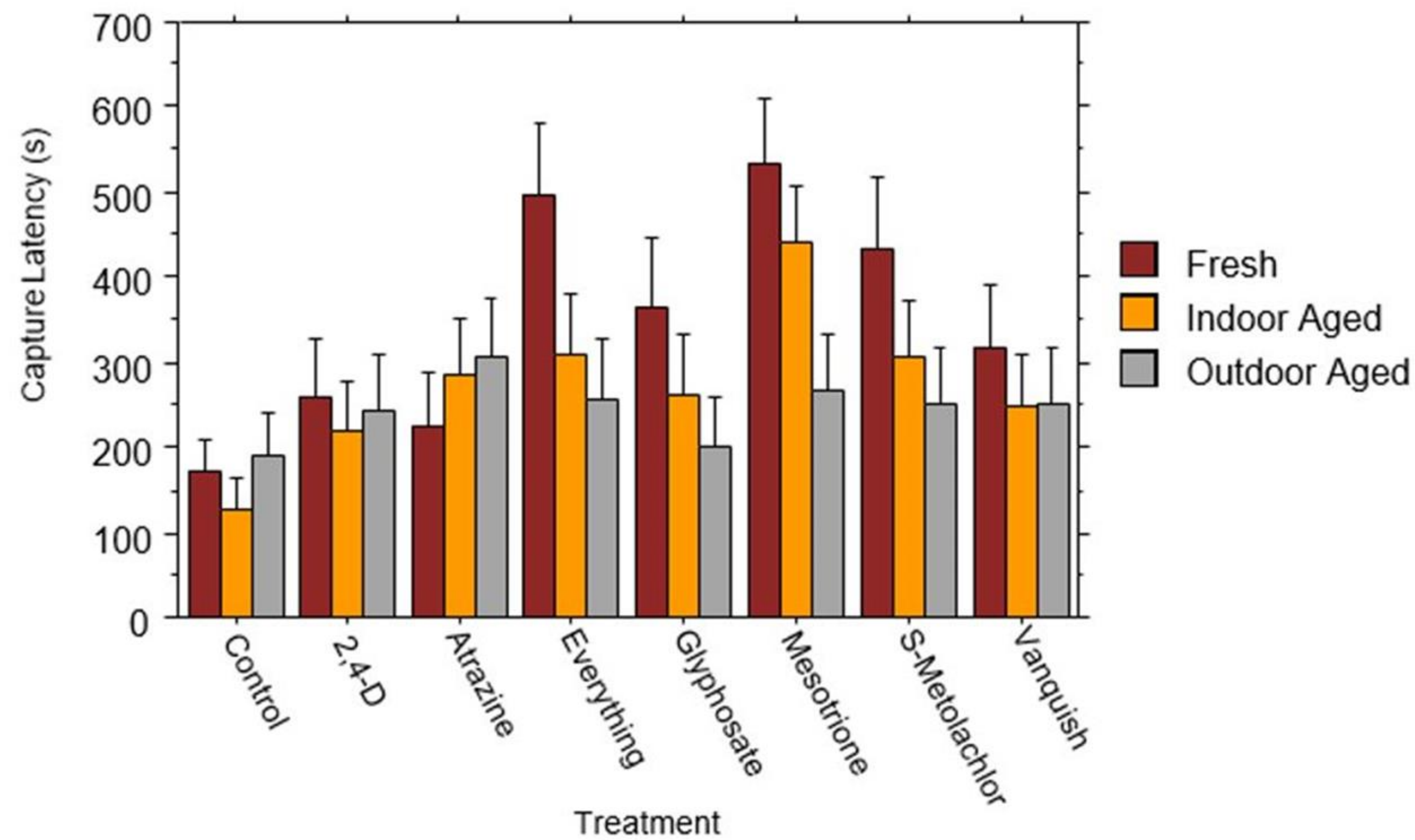
Table 3: Two-way within-between subjects ANOVA for the effects of herbicide and time on the weight of spiders within the outdoor-aged herbicide treatment.

Methods

Weight by time and herbicide treatment for Outdoor Aged soil



Capture latency after two weeks



Number of molts across herbicide and soil aged treatments